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(54) Method for screening fungicides utilizing dihydro orotate dehydrogenase (DHOD) inhibitor.

(57) Dihydroorotate dehydrogenase (DHOD) inhibition assays are utilized in a method for identifying new plant fungicides.

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Background of the Invention

Dihydroorotate dehydrogenase (DHOD) is an enzyme in the *de novo* pyrimidine biosynthetic pathway. Certain anticancer compounds have been reported as having inhibition of DHOD as their mode of action. *Biochemical Pharmacology*, Vol. 40, No. 4, pp. 709-714, 1990.

Phenoxyquinolines are a novel class of plant fungicides described in detail in U.S. Patent No. 5,145,843, European Patent Application 326330, and laid open Japanese application 246263/1989. One compound in this series is 8-chloro-4-(2-chloro-4-fluorophenoxy)quinoline (CCFQ). CCFQ completely inhibits the fungal pathogens Botrytis cinerea and Penicillium digitalum at 50 μg/ml in greenhouse tests. The fungicide has an IC_{50} (concentration that inhibits growth by 50%) of 0.15 μg/ml against Aspergillus nidulans. The fungi Cephalosporium and Neurospora are also sensitive to CCFQ.

We have discovered that the fungicidal activity exhibited by at least some of the phenoxyquinolines, including CCFQ, is a result of their inhibition of DHOD. This is a previously undescribed mode of action for fungicides.

Summary of the Invention

One aspect of the present invention is a method for identifying potential fungicides which comprises:

(a) testing a candidate compound in a DHOD inhibition assay, and

(b) if the candidate compound is active in the DHOD inhibition assay, testing the compound for activity against fungi.

Another aspect of the invention is a method for identifying potential plant fungicides which comprises testing a candidate compound in a DHOD inhibition assay wherein the assay utilizes DHOD isolated from any of the following plant pathogens: *Pyrenophora teres, Rhynchosporium secalis, Venturia inequalis, Alternaria mali, Rhizoctonia solani, Fusarium sp., Leptosphaeria nodorum, Pseudocercosporella herpotrichoides, Pyricularia oryzae, Pyricularia grisea, Phytophthora infestans, Phytophthora sp.,Pythium sp., Mycosphoerella tritici, Gorticium sasakii.*

Another aspect of the invention is a fungicide method which comprises applying a fungi inhibiting amount of a DHOD inhibitor to the locus it is desired to protect from fungi.

A further aspect of the invention is a fungicide composition comprising a DHOD inhibitor as the active ingredient in combination with a carrier.

Yet another aspect of the invention is a fungicide composition comprising a DHOD inhibitor in combination with another fungicide.

Detailed Description of the Invention

The physiological and biochemical responses caused by the experimental fungicide CCFQ are not characteristic of known modes of action. Treatment of *Aspergillus nidulans* with CCFQ perturbs cell wall synthesis in a non-specific manner, but causes no significant changes in respiration, lipid composition, amino acid content, or protein accumulation. It causes an unusual effect on synthesis of nucleic acids in that incorporation of radiolabelled precursors is stimulated by treatment with CCFQ. The nature of the mode of action for CCFQ was first suggested by growth remediation experiments. Some media supplements overcame growth inhibition by CCFQ. However, only a combination of uracil and a scavenger of free radicals, such as α-tocopherol, gave complete reversal. All these observations are consistent with the primary target of CCFQ being an electron-transferring enzyme in the pyrimidine biosynthetic pathway. The only such enzyme, DHOD, is in fact extremely sensitive to CCFQ in *in vitro* tests. Moreover, mutant *Aspergillus nidulans* strains that are resistant to CCFQ have been isolated, and the DHOD produced by these strains is resistant to inhibition by CCFQ. Finally, the DNA sequence responsible for imparting resistance to these mutant strains was isolated and was shown to comprise a mutation of the DHOD gene. These results establish DHOD inhibition as the mode of action for CCFQ. This is a novel fungicidal mode of action, and its discovery opens the opportunity to detect novel chemical compounds that attack the same target.

Our method for identifying potential fungicides does not require use of any particular DHOD inhibition assay. A suitable assay is described hereinafter, but those skilled in the art can readily substitute functionally equivalent test methods. For example, although the assay described hereinafter uses DHOD produced by *Pyricularia grisea*, the DHOD produced by other fungi may be substituted. Preferably the DHOD of a commercially significant target fungus, such as *Pyrenophora teres, Rhynchosporium secalis, Venturia inequalis, Alternaria mali, Rhizoctonia solani, Fusarium sp., Leptosphaeria nodorum, Pseudocercosporella herpotrichoides, Pyricularia oryzae, Pyricularia grisea, Phytophthora infestans, Phytophthora sp.,Pythium sp., Mycosphoerella tritici, Gorticium sasakii.*, is used. Compounds that are active in the DHOD inhibition assay are then tested using any desired fungicide activity test. In this context, "active in the DHOD inhibition assay" means that a measurable reduction in DHOD activity is observed. We have found it convenient in our work to restrict further testing to those compounds that cause at least a 50% reduction in DHOD activity.

Our fungicide method and fungicide composition require use of a "DHOD inhibitor". As used in describing and claiming the fungicide method and fungicide

composition, the term "DHOD inhibitor" encompasses any compound that: (a) produces measurable inhibition in a DHOD inhibition assay using DHOD from a target fungus; (b) is not a general enzyme inhibitor, and (c) is not a 4-substituted quinoline, quinazoline, cinnoline, naphthyridine, pyridopyrimidine, thienopyrimidine, furopyrimidine, or other compound that was known to have fungicidal activity prior to the filing date of this application. It should be understood that no fungicide was known to have DHOD inhibition as its mode of action at the time the present invention was made. Applicants invention is not intended to encompass fungicidal use of any previously known fungicide.

Preferred DHOD inhibitors are those which produce at least a measurable reduction in DHOD activity when tested at 10μg/mL in the *Pyricularia grisea* DHOD Inhibition Assay described hereinafter.

More preferred DHOD inhibitors are those which produce at least a 25% reduction in DHOD activity when tested at 10μg/mL in the *Pyricularia grisea* DHOD Inhibition Assay.

Especially preferred DHOD inhibitors are those which produce at least a 50% reduction in DHOD activity when tested at 10μg/mL in the *Pyricularia grisea* DHOD Inhibition Assay.

R. W. Miller has described DHOD isolation and assay methods. *Can. J. Biochem.* 53, 1288 (1975); *Methods in Enzymology,* 51, 63 (1978). The following DHOD inhibition assay is adapted from those described by Miller.

Pyricularia grisea DHOD Inhibition Assay

DHOD inhibition is measured by spectrophotometrically observing (at 610 nm) the reduction of dichloroindophenol (DCIP) by electrons liberated when dihydroorotate is oxidized to orotate. Electrons are transferred to DCIP in the reaction mixture via ubiquinone-30. DCIP is a dark blue dye that strongly absorbs at 610 nm and becomes clear when reduced. Rotenone and KCN are added to the reaction mixture to prevent transfer of electrons to DCIP via other pathways.

"Stock reaction mix" for use in the DHOD inhibition assay comprises the following reagents in solution in T-TX100 buffer ("T-TX100 buffer" comprises 50mM Tris·HCl, pH 7.5 containing 0.1% Triton X100):

ubiquinone-30	500 μM
dihydroorotic acid	500 μM
dichloroindophenol (DCIP)	250 μM
potassium cyanide (KCN)	43.75 mM
rotenon	150 μM

"stock control mix" is identical to stock reaction mix, except that the ubiquinone-30 is omitted.

DHOD for use in the assay was obtained as follows. Cultures of *Pyricularia grisea* Sacc. strain P-2 (ATCC 58061, Tokousbalides and Sisler, 1979) were maintained on oatmeal agar, which was prepared by straining 37.5 g of oatmeal boiled in 1 liter of water through two layers of cheesecloth, then adding 13 g of agar per liter before autoclaving 30 minutes. Oatmeal agar plates were flood-inoculated with conidia or macerated mycelia and incubated at 24°C for ten days under cool white fluorescent lighting. Conidia were harvested by dislodging with a sterile glass rod in water containing 0.1% Tween 20, then filtered through sterile glass wool. Flasks containing Media 56 (Coursen and Sisler, *Amer. J. Bot.* 47, 541 (1960)) supplemented with 2 x phosphate and 2% yeast extract were inoculated to provide 1.5 X 10^7 conidia L^{-1}. The cultures were incubated at 150 rpm for 38-42 hours at 30°C. Mycelia were collected on miracloth by vacuum filtration and washed twice with cold Tris buffer (50 mM Tris·HCl, pH 7.5).

Harvested mycelia were resuspended in two volumes of Tris buffer. Equal volumes (175 mL) of resuspended mycelia and 0.5 mm diameter glass beads were placed in a 350 mL Beadbeater ™ chamber (Biospec Products, Bartlesville, OK), Mycelia were broken by operating the Beadbeater for four 30-second cycles with 30-second cooling intervals between cycles. The homogenate was decanted, and the beads washed with approximately one volume of buffer which was added to the homogenate. The pooled homogenate was centrifuged at 3,000 x g for 10 min at 4°C. The supernatant was recovered and adjusted to 25 mM $CaCl_2$ by slowly adding solid $CaCl_2 \cdot H_2O$. The supernatant was then centrifuged at 30,000 x g for 30 minutes at 4°C. The pelleted material was homogenized in a glass/teflon tissue grinder with approximately 150 mL of T-TX100 buffer per liter of pooled homogenate. The homogenate was frozen dropwise in liquid N_2 and stored at minus 80°C.

"Dilute DHOD solution" was prepared by diluting one volume of homogenate with four volumes of T-TX100 buffer containing 6.25 mM dihydroorotate. Using this dilution rate gives an activity in the assay of 50-70 $mAbs_{610nm}$/min for 5 minutes. The presence of the dihydroorotate is required to maintain satisfactory stability of the enzyme.

Samples (2-3 mg) of compounds to be screened are dissolved in DMSO to obtain a stock sample having a concentration of 1 mg/mL for each compound.

The assay is preferably conducted using Falcon® 96-well, flat bottom polystyrene plates. These plates have 96 wells arrayed in 12 columns and 8 rows. To each well in columns 2, 5, and 8 of the 96 well plate is added 20 μL of a different stock sample. Accordingly, 24 different compounds can be tested on one plate. Then 180 μL of T-TX-100 buffer is added to

each of the wells in columns 2, 5, and 8, resulting in a 10-fold dilution from the initial concentration of the stock sample.

To each well in columns 3,4,6,7,9, and 10 of the plate is added 80 μL of stock reaction mix. Then, for each row, 20 μL of diluted test solution from the well in column 2 are transferred to each of the two wells in columns 3 and 4 of the same row. The contents of each well are mixed. Similarly, for each row, 20 μL of diluted test solution from the well in column 5 are transferred to each of the two wells in columns 6 and 7 , and 20 μL of diluted test solution from the well in column 8 are transferred to each of the two wells in columns 9 and 10. At this point, each of the wells in columns 3,4,6,7,9, and 10 contains 100 μL of solution with a concentration of 20 μg/mL of test compound. Columns 3 and 4 contain duplicate samples for the first eight compounds, columns 6 and 7 contain duplicate samples for the second eight compounds, and columns 9 and 10 contain duplicate samples for the remaining eight compounds.

The inhibition of DHOD by CCFQ may desirably be used to standardize the effects of other test compounds on DHOD activity. For this purpose, we typically include CCFQ at concentration of 10, 5, and 1 μg/mL in DMSO (resulting in final concentration of 0.1, 0.05, and 0.01 μg/mL) as the last three compounds on the plate.

The wells in column 1 of the plate are used for determining background and total activity. 20 μL of T-TX100 buffer is pipetted into each well in column 1 of the plate. Then, to each well in the first four rows (A,B,C, and D) of column 1 are added 80 μL of stock control mix (background). To each well in the last four rows of column 1 (E,F,G,and H) are added 80 μL of stock reaction mix (total).

Preparation of the plates is preferably automated, using a Biomek 1000 Automated Laboratory Workstation (Beckman) to dilute the stock compounds and add appropriate volumes of reaction solutions and compounds to the individual wells of the 96-well microtiter plate.

The assay is initiated by adding 100 μL of dilute DHOD solution to each well, which is conveniently done using an Eppendorf 8-channel dispenser. After this solution is added, the concentration of test compound in each well is 10 μg/mL. The contents of each well on the plate is mixed, and changes in absorbance at 610 nm are recorded every 10 seconds for 5 minutes using the THERMO_{max}™ (Molecular Devices) plate reader(set at 30°C incubation temperature).

The rate of absorbance change per minute (mAbs_{610nm}/min) due to reduction of DCIP is then calculated for each sample and the background controls. A plot of absorbance versus time for each well yields a downward sloping line, reflecting decreased absorbance as the DCIP is reduced. Under the conditions of the assay described above, the plot is essentially linear. Compounds that inhibit DHOD reduce the reaction rate and result in a linear plot with a reduced slope. Percent activity is calculated using the following formula

$$\%\ \text{activity} = 100 \times \frac{(\text{rate}_{test} - \text{rate}_{background})}{(\text{rate}_{total} - \text{rate}_{background})}$$

where $\text{rate}_{background}$ is given by the slope of the curve obtained for cells in the first four wells of column 1, rate_{total} refers to the slope of the curve obtained for cells in the second four rows of column 1, and rate_{test} refers to the slope obtained for the wells containing test compound.

We have found a strong correlation between activity of a compound in the DHOD assay and fungitoxicity.

The present invention is directed to fungicidal use of compounds that inhibit DHOD, as opposed to fungicidal use of compounds that inhibit enzymes generally. An example of a compound that inhibits enzymes generally is maneb. To eliminate the possibility that a compound active in the DHOD assay is a general enzyme inhibitor, the active compound can be tested in a second enzyme assay. A suitable assay for this purpose is the *E. Coli* alkaline phosphatase assay described by Garen and Levinthal, *Biochim. Biophys. ACTA, 38*, 470 (1960). If the compound is not active in the second enzyme assay, it may be concluded that the compound does not inhibit enzymes generally.

Biological efficacy of a fungicidal compound in whole organisms is influenced by many factors, including not only intrinsic activity of the compound, i.e. efficiency of its interaction with the target molecule, but also stability of the compound and ability of the compound to be translocated to the target site. The DHOD inhibition assay measures the intrinsic activity of the compound. It will be appreciated by those skilled in the art that once a potential fungicide is detected using the DHOD assay, conventional techniques must be used to determine the usefulness of the compound in various environments.

As used herein, the term "fungi inhibiting amount of DHOD inhibitor" refers to an amount of DHOD inhibitor sufficient to kill or inhibit the fungi it is desired to control.

When employed in the treatment of plant fungal diseases, the DHOD inhibitors are applied to the plants in a disease inhibiting and phytologically acceptable amount. The term "disease inhibiting and phytologically acceptable amount," as used herein, refers to an amount of a compound of the invention which kills or inhibits the plant disease for which control is desired, but is not significantly toxic to the plant. This amount will generally be from about 1 to 1000 ppm, with 1 to 500 ppm being preferred. The exact concentration of compound required varies with the fungal disease to be controlled, the type formulation

employed, the method of application, the particular plant species, climate conditions and the like. A suitable application rate is typically in the range from 100 to 1000 g/ha The compounds of the invention may also be used to protect stored grain and other non-plant loci from fungal infestation.

Compositions

When used on plants, DHOD inhibitors are applied in the form of compositions which are important embodiments of the invention, and which comprise a DHOD inhibitor as active ingredient in combination with a phytologically-acceptable inert carrier. The compositions are either concentrated formulations which are dispersed in water for application, or are dust or granular formulations which are applied without further treatment. The compositions are prepared according to procedures and formulae which are conventional in the agricultural chemical art, but which are novel and important because of the presence therein of the compounds of this invention. Some description of the formulation of the compositions will be given, however, to assure that agricultural chemists can readily prepare any desired composition.

The dispersions in which the compounds are applied are most often aqueous suspensions or emulsions prepared from concentrated formulations of the compounds. Such water-soluble, water-suspendable or emulsifiable formulations are either solids usually known as wettable powders, or liquids usually known as emulsifiable concentrates or aqueous suspensions. Wettable powders, which may be compacted to form water dispersible granules, comprise an intimate mixture of the active compound, an inert carrier and surfactants. The concentration of the active compound is usually from about 10% to about 90% by weight. The inert carrier is usually chosen from among the attapulgite clays, the montmorillonite clays, the diatomaceous earths, or the purified silicates. Effective surfactants, comprising from about 0.5% to about 10% of the wettable powder, are found among the sulfonated lignins, the condensed naphthalenesulfonates, the naphthalenesulfonates, the alkylbenzenesulfonates, the alkyl sulfates, and nonionic surfactants such as ethylene oxide adducts of alkyl phenols.

Emulsifiable concentrates of the compounds comprise a convenient concentration of a compound, such as from about 50 to about 500 grams per liter of liquid, equivalent to about 10% to about 50%, dissolved in an inert carrier which is either a water miscible solvent or a mixture of water-immiscible organic solvent and emulsifiers. Useful organic solvents include aromatics, for example the xylenes, and the petroleum fractions, especially the high-boiling naphthalenic and olefinic portions of petroleum such as heavy aromatic naphtha. Other organic solv nts may also be used, such as the terpenic solvents including rosin derivatives, aliphatic ketones such as cyclohexanone, and complex alcohols such as 2-ethoxyethanol. Suitable emulsifiers for emulsifiable concentrates are chosen from conventional nonionic surfactants, such as those discussed above.

Aqueous suspensions comprise suspensions of water-insoluble compounds of this invention, dispersed in an aqueous vehicle at a concentration in the range from about 5% to about 50% by weight. Suspensions are prepared by finely grinding the compound, and vigorously mixing it into a vehicle comprised of water and surfactants chosen from the same types discussed above. Inert ingredients, such as inorganic salts and synthetic or natural gums, may also be added, to increase the density and viscosity of the aqueous vehicle. It is often most effective to grind and mix the compound at the same time by preparing the aqueous mixture, and homogenizing it in an implement such as a sand mill, ball mill, or piston-type homogenizer.

The compounds may also be applied as granular compositions, which are particularly useful for applications to the soil. Granular compositions usually contain from about 0.5% to about 10% by weight of the compound, dispersed in an inert carrier which consists entirely or in large part of clay or a similar inexpensive substance. Such compositions are usually prepared by dissolving the compound in a suitable solvent, and applying it to a granular carrier which has been pre-formed to the appropriate particle size, in the range of from about 0.5 to 3 mm. Such compositions may also be formulated by making a dough or paste of the carrier and compound, and crushing and drying to obtain the desired granular particle size.

Dusts containing the compounds are prepared simply by intimately mixing the compound in powdered form with a suitable dusty agricultural carrier, such as kaolin clay, ground volcanic rock and the like. Dusts can suitably contain from about 1% to about 10% of the compound.

It is equally practical, when desirable for any reason, to apply the compound in the form of a solution in an appropriate organic solvent, usually a bland petroleum oil, such as the spray oils, which are widely used in agricultural chemistry.

Fungicides are generally applied in the form of a dispersion of the active ingredient in a liquid carrier. It is conventional to refer to application rates in terms of the concentration of active ingredient in the carrier. The most widely used carrier is water.

DHOD inhibitors can also be applied in the form of an aerosol composition. In such compositions the active compound is dissolved or dispersed in an inert carrier, which is a pressure-generating propellant mixture. The aerosol composition is packaged in a container from which the mixture is dispensed through an atomizing valve. Propellant mixtures com-

prise either low-boiling halocarbons, which may be mixed with organic solvents, or aqueous suspensions pressurized with inert gases or gaseous hydrocarbons.

Claims

1. A method for identifying potential fungicides which comprises:
   (a) testing a candidate compound in a DHOD inhibition assay, and
   (b) if the candidate compound is active in the DHOD inhibition assay, testing the compound for activity against fungi.

2. The method of claim 1 including the additional step of, if the candidate compound is active in the DHOD inhibition assay, testing the candidate compound for activity against a second enzyme to eliminate the possibility that the compound is a general enzyme inhibitor.

3. The method of claim 2 wherein the second enzyme is *E. coli* alkaline phosphatase.

4. A method as claimed in any one of the preceding claims for identifying potential plant fungicides, wherein the assay utilizes DHOD isolated from any of the following plant pathogens: *Pyrenophora teres, Rhynchosporium secalis, Venturia inequalis, Alternaria mali, Rhizoctonia solani, Fusarium sp., Leptosphaeria nodorum, Pseudocercosporella herpotrichoides, Pyricularia oryzae, Pyricularia grisea, Phytophthora infestans, Phytophthora sp.,Pythium sp., Mycosphoerella tritici, Gorticium sasakii.*

5. The method of any one of claims 1 to 4, wherein the DHOD inhibition assay utilizes DHOD produced by *Pyricularia grisea*.

6. The use as a fungicide of a DHOD inhibitor.

7. The use in accordance with claim 6 wherein the DHOD inhibitor is one which produces a measurable reduction in DHOD activity when tested in the *Pyricularia grisea* DHOD Inhibition Assay at 10μg/mL.

8. The use in accordance with claim 7 wherein the DHOD inhibitor is one which produces at least a 25% reduction in DHOD activity when tested in the *Pyricularia grisea* DHOD Inhibition Assay at 10μg/mL.

9. The use in accordance with claim 8 wherein the DHOD inhibitor is one which produces at least a 50% reduction in DHOD activity when tested in the *Pyricularia grisea* DHOD Inhibition Assay at 10μg/mL.

10. A fungicide composition comprising a DHOD inhibitor as the active ingredient in combination with a carrier and/or a second fungicide.

11. A fungicide composition of claim 10 wherein the DHOD inhibitor is one which produces a measurable reduction in DHOD activity when tested at 10μg/mL in a DHOD inhibition assay using DHOD isolated from any of the following plant pathogens: *Pyrenophora teres, Rhynchosporium secalis, Venturia inequalis, Alternaria mali, Rhizoctonia solani, Fusarium sp., Leptosphaeria nodorum. Pseudocercosporella herpotrichoides, Pyricularia oryzae, Pyricularia grisea, Phytophthora infestans, Phytophthora sp.,Pythium sp., Mycosphoerella tritici, Gorticium sasakii.*

12. The fungicide composition of claim 11 wherein the DHOD inhibitor is one which produces a measurable reduction in DHOD activity when tested at 10μg/mL in the *Pyricularia grisea* DHOD Inhibition Assay .

13. The fungicide composition of claim 12 wherein the DHOD inhibitor is one which produces at least a 25% reduction in DHOD activity when tested in the *Pyricularia grisea* DHOD Inhibition Assay at 10μg/mL.

14. The fungicide composition of claim 13 wherein the DHOD inhibitor is one which produces at least a 50% reduction in DHOD activity when tested in the *Pyricularia grisea* DHOD Inhibition Assay at 10μg/mL.

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EUROPEAN SEARCH REPORT

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,X, D	US-A-5 145 843 (WENDELL R. ARNOLD; MICHAEL J. COGHLAN; GLENN P. JOURDAN ET AL.) 8 September 1992 * column 11; example 35; table 1 * * column 20, line 10 - line 68 * * example 35; table 6 * * example 35; table 7 * * column 29, line 15 - column 30, line 46 * * column 34, line 30 - line 53 * * claims 1,2,20,25,26 *	6-14	C12Q1/32 A01N43/42
Y	HELMWARD ZOLLNER 'HANDBOOK OF ENZYME INHIBITORS' 1989 , VCH VERLAGSGESELLSCHAFT MBH , WEINHEIM (DE) * page 71 *	6-14	
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	26 MAY 1993	DöPFER K.P.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	CHEMICAL ABSTRACTS, vol. 69, no. 19, 4 November 1968, Columbus, Ohio, US; abstract no. 75858, WELVAERT, W. 'Control of Fusarium oxysporum on Aechmea fasciata' page 7075 ; * abstract * & MEDED. RIJKSFAC. LANDBOUWWTENSCH., GENT 1967, 32(3-4), 459-65 -----	6-14	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

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THE HAGUE	26 MAY 1993	DöPFER K.P.

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X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

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